DiM: Prognostic Score for Second- or Further-line Immunotherapy in Advanced Non–Small-Cell Lung Cancer: An External Validation

DiM: prognostic score for second or further-line immunotherapy in advanced non-small-cell lung cancer: an external validation.

Arsela Prelaj[1*], Giuseppe Lo Russo[1*], Claudia Proto[1], Diego Signorelli[1], Roberto Ferrara[1], Giulia Galli[1], Alessandro De Toma[1], Giovanni Randon[1], Filippo Pagani[1], Benedetta Trevisan[1], Monica Ganzinelli[1], Nicoletta Zilembo[1], Michele Montrone[2], Vito Longo[2], Francesco Pesola[2], Pamela Pizzutilo[2], Gabriella Del Bene[2], Niccolò Varesano[2], Domenico Galetta[2], Valter Torri[3], Marina Chiara Garassino[1], Massimo Di Maio[4], Annamaria Catino[2].

* These authors contributed equally.

[1]Medical Oncology Department, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy;
[2]Medical Thoracic Oncology Unit, IRCCS Oncologico Giovanni Paolo II of Bari, Italy;
[3]Pharmacological Research Institute IRCSS Mario Negri, Via La Masa 19, 20156 Milano, Italy.
[4]Department of Oncology, University of Turin, at Medical Oncology, Mauriziano Hospital, Torino, Italy;

Corrisponding Author: Arsela Prelaj: arsela.prelaj@istitutotumori.mi.it, Telephone: +39 3292187197, Fax: +39 0223903647, Medical Oncology Department, Fondazione IRCCS Istituto Nazionale Tumori, Via Giacomo Venezian, 1, 20133 Milan, Italy.

Background

Beyond PD-L1 value, oncologists can only use clinical characteristics to candidate advanced non-small-cell lung cancer (aNSCLC) patients for immunotherapy.

A clinical prognostic score composed by ECOG-PS, sex, histology, stage, use of platinum-based therapy at first-line and response to first-line has categorized 3-different prognostic groups for patients treated with second-line chemotherapy. The study aim is to validate the same score in aNSCLC patients treated with second or further-line immunotherapy.

Methods

We collected data from two Italian centers. A score was generated which divided patients into 3-prognostic-groups: best (B:<5), intermediate (I:5-9), worst (W:>9). Overall survival (OS) and progression-free survival (PFS) were the endpoints.

Results

347 patients were included for analysis. Median age was 66 years (30 – 88y), most were <70 years/67.5%, male/70.7%, smokers/79.5% and adenocarcinoma/74.6%. ECOG-PS was: 0(23%), 1(54.5%) and 2(22.5%). Patients distribution was: 28%/51%/21% in the B/I/W groups, respectively. mOS was 18.0 months for best, 8.5 months for intermediate (HR vsB 1.83, 95%CI 1.35 – 2.47, p<0.001) and 2.6 months for worst group (HR vsB 5.77, 95%CI 3.99 – 8.33, p<0.001). mPFS was 3.4 months for best, 3.7 months for intermediate (HR vsB 1.35, 95% CI 1.03 – 1.77, p=0.032) and 1.9 months for worst group (HR vsB 2.51. 95% CI 1.80- 3.50, p<0.001).

Conclusions

This prognostic score, is able to predict outcome in aNSCLC patients treated with immunotherapy.

The worst category has a dismal life expectancy, and probably would not benefit from any active systemic therapy. Reasonably, for these patients, best supportive care could be the best choice.

Keywords: non-small cell lung cancer; biomarker; prognostic score; predictive score; immunotherapy; second-line;

1. Introduction

Immune checkpoint inhibitors (ICIs) have significantly changed the therapeutic landscape of advanced non-small cell lung cancer (aNSCLC) [1]. Currently, pembrolizumab is the standard of care as first-line therapy in programmed-death ligand-one (PD-L1) $\geq$50% NSCLC (KEYNOTE-024) [2,3]. Nivolumab and pembrolizumab have been the first two ICIs approved on the basis of a significant improvement in overall survival (OS) versus docetaxel in pre-treated aNSCLC patients (pts), in both squamous and non-squamous histology [4-6]. Atezolizumab is another therapeutic option in the same setting [7], as well as durvalumab demonstrated activity even in strongly pre-treated aNSCLC pts [8].

Despite improvement in survival, only a limited number of pts respond to immunotherapy and even less pts experience a durable response [6]. The progression-free survival (PFS) and OS curves in the pivotal second-line (2L) studies with nivolumab, pembrolizumab and atezolizumab are overlapping in the first months (mo) of therapy demonstrating that most patients do not respond to ICIs, with a non-negligible risk of early clinical failure [4-8]. The discovery of predictive and prognostic biomarkers remains a hot topic.

PD-L1 expression is the only approved biomarker in aNSCLC patients; however multiple studies reported several biological and technical limitations, due to the temporal changes in PD-L1 expression and its intra-tumoural heterogeneity [9]. Contrasting results were described in a proportion of patients responding to ICIs with low or negative PD-L1 [6,10], thus its negative predictive role remains suboptimal [11]. Beyond PD-L1, nowadays oncologists can only use clinical characteristics to candidate or not patients for ICIs therapy. [12-15]. Recently, other potential biomarkers have been investigated, such as tumour mutation burden, immune-score, CD8-positive tumour-infiltrating lymphocytes and immune gene signature, with interesting results [16]. However, to date, none of these factors has gained a definite role in clinical practice [16].

A crucial task is also played by cancer-associated inflammation, which is correlated with worse outcome [17,18]. Moreover, different peripheral blood parameters have been investigated in various malignancies [11,19]. Blood inflammatory biomarkers correlated with scarce therapeutic response and poor prognosis to conventional treatments [17] and demonstrated an association with survival outcome in advanced melanoma patients receiving ICIs [20,21]. Their prognostic role has been reported also in aNSCLC patients treated with ICIs [22,23,24]. However, few evidences exist regarding the predictive role of peripheral blood biomarkers in aNSCLC treated with anti-PD-(L)-1 inhibitors [22-28].

Formerly, a clinical prognostic score (DiM score), composed by Eastern Cooperative Oncology Group Performance Status (ECOG-PS), sex, histology (squamous, adenocarcinoma, other histology), stage (IIIB or IV), use of platinum-based therapy at first-line and type of response to first-line (complete or partial response, no response), categorized 3 different prognostic groups for patients treated with second-line chemotherapy (CHT) [29]. The score was developed using individual data of 1197 patients enrolled in 9 randomized trials of 2L CHT.

Afterwards, DiM score has been externally validated in 551 patients enrolled in a randomized phase III trial comparing vinflunine with docetaxel in the same setting, confirming its prognostic importance [30].
The aim of this study is to assess if DiM score, developed and validated in pts receiving CHT, is able to discriminate also the outcome of aNSCLC patients treated with second or further-line IO, in order to identify patient who are less likely to respond and potentially helping decision making.

2. Materials and Methods

This study was conducted at the Fondazione IRCCS Istituto Nazionale Tumori of Milan and at the IRCCS Oncologico Giovanni Paolo II of Bari, Italy. Accomplished in accordance with the Declaration of Helsinki, Good Clinical Practice and local ethical guideline. All enrolled alive patients signed informed consent.

2.1 Study population

From August 2015 to December 2018 we conducted a retrospective bicentric study of 347 consecutive advanced NSCLC patients receiving single-agent anti-PD-(L)-1 inhibitors in 2nd or further-line therapy [193 from Milan (C1) and 154 from Bari (C2)].
Eligible patients fulfilled the following inclusion criteria: cytological/histological diagnosis of aNSCLC, pre-treated patients (relapsed or stage IIIB to IV) that had received at least one infusion of anti PD-(L)-1 agent as a second or further-line therapy. Patients who performed ICIs within a clinical study were also included.
Exclusion criteria comprised immunotherapy as first-line or in combination with other systemic drugs.
Clinical characteristics including also treatment information for both centres are reported in Table 1.

2.2 Treatment

ICIs was administered intravenously (IV); Nivolumab initially at a dose of 3 mg/kg and later, since May 2018, at a fixed dose of 240 mg every 2 weeks; Pembrolizumab at a dose of 2 mg/kg every 3 weeks in PD-L1≥1% patients, Atezolizumab at a fixed dose of 1200 mg every 3 weeks and Durvalumab at a dose 10 mg/kg every 2 weeks. The treatment was continued until the occurrence of disease progression, unacceptable toxicity, withdrawal, or death. Treatment beyond progressive disease (PD) was permitted, if there was a clinical benefit according to clinician's judgement.

2.3 Statistical analysis

The primary endpoint OS, and its association with the prognostic score, in order to identify potential poor prognostic groups who are less likely to obtain a favourable outcome with ICIs. OS was calculated from the start of ICIs treatment until the time of death or the last follow-up.

Secondary endpoints were PFS and its association with prognostic score. PFS was calculated from the date of first ICI administration until disease progression or death due to any cause, or the last follow-up visit for patients alive without disease progression. Kaplan–Meier method was used to calculate median PFS (mPFS) and median OS (mOS) with their 95% confidence interval, and to generate survival curves for PFS and OS divided for three different risk categories. Median follow-up was calculated according to the inverted Kaplan-Meier technique [31]. Log-rank test (Mantel-Cox) was used to evaluate statistical differences in PFS and OS, which was defined at $p<0.05$ level. The prognostic score was divided using the scoring system presented in Table 2. Similar to previous studies, patients were split in three different groups with a cut-off well balanced along the range of values: <5, 5-9, >9 for best, intermediate and worst category, respectively. Cox proportional hazard model was used to compare the three categories. The concordance C-index was calculated using the model proposed by Pencina et al. to measure the power of discriminations [32,33]. All statistical analyses were

performed using the Statistical Package for the Social Sciences (SPSS) program version 25.0 (IBM, Armonk, NY) and R 3.5.1 [34]

2.4 Response evaluation

Radiological assessments consisted in a baseline total body computed tomography (TB CT) scan, subsequently performed every 3-4 cycles, or whenever progression disease was clinically suspected.
Tumour response was assessed according to Response Evaluation Criteria in Solid Tumours (RECIST) v.1.1 criteria [35]. Overall response rate (ORR) was defined as a sum of complete (CR) and partial (PR) response, while disease control rate (DCR) as the sum of CR+PR and stable disease (SD). Patient with PD who maintained a clinical benefit according to clinician's judgement were treated beyond progression and were considered as SD if PD was not confirmed.

3. Results

3.1 Patients' characteristics

Three hundred forty-seven aNSCLC patients treated with anti-PD-(L)-1 in second or further-line therapy were included in the analysis. Patients' characteristics are summarised in Table 1.
Two hundred and forty-six patients were male (70.7%) and 276 were smokers (79.5%), median age was 66 years (range 30-88 years) and 113 (32.5%) were older than 70 years. Median ECOG-PS was 1 (range 0-1) with an ECOG-PS 2 in 22.5% of patients. The histological sub-types were adenocarcinoma 65.4%, squamous cell 31.5% and other histologies 3.1%. At baseline, bone, liver and brain metastases were present in 38.3%, 19.6% and 18.4% of patients, respectively. Two thirds of patients (64.3%) received ICIs in second-line, while 124

patients received anti-PD-(L)-1 therapy in ≥3rd line.

3.2 General response and overall survival outcomes

All 347 patients included in the study were assessable for survival analysis.

At the time of data cut-off (December 2018), 306 patients (88%) had disease progression and 260 patients had died (75%). Overall, after a median follow-up of 29 mo [95% confidence interval (CI) 25.2 – 34.6 mo] mPFS was 3.1 mo (95% CI 2.6 – 3.5 mo) and mOS was 7.6 mo (95%CI 5.7 – 9.5 mo). ORR and disease control rate DCR were 16.2% (95%CI 12.6 – 20.4) and 44.1% (95%CI 39.0 – 49.4), respectively.

3.3 Prognostic Index

The index score was assigned and calculated based on the proposed scoring system from previous publications from Di Maio et al. (Table 2) [23-24]. The patients were divided into three different categories: 96 pts (27.7%) had a low score < 5 (best category: B), 178 (51.3%) obtained a score between 5 and 9 (intermediate category: I) and the remaining 73 pts (21%) received a high score >9 (worst category: W).

3.4 Survival results among groups

Median OS was 18.0 mo (95%CI 11.1 – 24.8 mo) for the B group, 8.5 mo (95%CI 6.6 – 10.3 mo) for the I group and 2.6 mo (95%CI 1.8 – 3.4 mo) for the W group. In the Figure 1 are presented the Kaplan-Meier curves according to the three prognostic groups (Fig. 1). We used the Cox hazard model to describe differences between the I vs B groups [hazard ratio (HR) 1.83, 95%CI 1.35 – 2.47, p<0.001] and W vs B groups (HR 5.77, 95%CI 3.99 – 8.33, p<0.001). The C-index of the model for OS was 0.67 (95%CI 0.63 – 0.70).

As secondary endpoint we evaluated PFS among three different categories. Median PFS was 3.4 mo (95%CI 2.1 – 4.7 mo), 3.7 mo (95%CI 3.2 – 4.2 mo) and 1.9 mo (95%CI 1.5 – 2.2 mo) for B, I and W category, respectively. Kaplan-Meier curves of PFS are reported in Figure 2. The Cox model showed that the difference was statistically significant when comparing I vs B groups (HR 1.35, 95% CI 1.03 – 1.77, p=0.032) and W vs B groups (HR 2.51. 95% CI 1.80- 3.50, p<0.001).

3.5 Multivariate analysis according to OS

A multivariate analysis according to OS for overall population was performed including baseline patient's characteristics (see Table nr.3) such as: age, gender, smoke, pack/year, ECOG PS, histology, stage, liver, brain or bone metastases, use of ICIs as second or further line, use of platinum-based therapy as first-line, ORR at first-line. Only ECOG PS (HR 0.14, IC95% 0.092 – 0.213 p<0.0001), liver metastases at baseline (HR 1.73, IC95% 1.27 – 2.36, p=0.001) and smoke expressed in pack/year (HR 0.71, IC95% 0.53 – 0.96, p=0.026) are confirmed as relevant prognostic factors.

3.6 Results stratifying the model by Institutions.

In order to identify differences between the two single Institutions we implemented separated survival analyses for OS and PFS for C1 and C2. Median OS was 7.6 mo (95%CI 5.4 – 9.9 mo) and 7.5 mo (95%CI 4.4 – 10.6 mo), respectively for C1 and C2 (p=0.761). Median PFS was 2.2 (95%CI 1.9 – 2.6 mo) and 3.8 mo (95%CI 2.6 – 3.5 mo), respectively for C1 and C2 (p=0.005). Kaplan Meier curves for PFS and OS are reported in the Appendix (see Supplement Figure 1 and 2).

4. Discussion

In this analysis, we showed that the DiM prognostic score, initially developed and validated in patients with advanced NSCLC receiving second-line chemotherapy, performs well also in patients receiving ICIs, allowing the

identification of patients with a good prognosis and, on the other hand, a subgroup of patients with very short life expectancy.

In recent years, immunotherapy has changed the survival landscape of aNSCLC, significantly prolonging mOS and offering an interesting chance of obtaining a long-term benefit in a minority of subjects. Nonetheless, only a small percentage of patients (18-20%) respond to ICIs in second-line with a mPFS around 2-4 mo [4-8].

The identification of prognostic and/or predictive clinical factors and biomarkers remains a crucial topic. Early identification of responders and non-responders to ICIs is decisive in the attempt of avoiding inadequate treatments and optimizing use of drugs in clinical practice, sparing unnecessary toxicity and costs, and is also essential to detect those patient who may experience the detrimental effect caused by the hyperprogressive disease [36-38]. Different clinical factors are currently under investigation such as ECOG-PS, age, smoking status, hyponatremia, use of steroids and antibiotics, the presence of liver, bone and brain metastases but there is no consensus regarding the ICIs benefit in these patients [39,40].

In both studies of Di Maio et al. of the development and validation of the DiM score, the authors classified patients into three different survival groups: B, I and W category. Their findings lead to the identification of a subset of patients (W category) with a bad prognosis. For those patients, in fact, mOS was shorter than 4 months. Although this represents a prognostic, and not predictive information, the authors concluded that the chance to benefit from active treatments was very small for this category of patients [29,30].

In order to indirectly compare our cohort of patients treated with ICIs with these patients treated with 2L CHT, we decided to perform an external validation of the score in the same setting. Therefore, we speculated that if the score is successfully validated in our series of patients treated with ICIs, these results could confirm its prognostic role.

The validation of the DiM score in 347 pts treated with ICIs also allowed to carry out an indirect comparison between patients treated with CHT and patients treated with ICIs.

Results from our study demonstrated that DiM score was able to permit a good patients distribution into three different survival categories, also in ICIs second-line treated patients. The C-index, according to OS, was good

(0.670) indicating a satisfactory discrimination according to the three risk categories.

In particular, patients within the W group had the shortest median OS and PFS with 2.6 and 1.9 mo, respectively. Probably these patients respond poorly to any anti-cancer therapy and best supportive care could be the best choose for them.

The differences on mPFS occurred between the two centres participating in the analysis can be explained probably due to the timing of radiologic assessment which was longer in the C2 compare to C1.

Similarly, to other papers [22-23,39-40], our study underlined the negative prognostic role of ECOG-PS 2, in ICIs-treated patients with aNSCLC.

Treatment of patients with poor ECOG-PS with ICIs remains an argument of clinical debate. As a matter of fact, patients with poor ECOG-PS have a poor prognosis, and are less likely to benefit from ICIs, probably due to their ineffective immune system with less functional lymphocytes. However, currently ongoing prospective trials are assessing the efficacy of ICIs (NCT02733159, NCT02879617) in poor PS patients, and will help to better define the role of ICIs in this setting [41,42]. Considering that these drugs are characterized by a favourable toxicity profile, many clinicians could be tempted to consider eligible for ICIs many patients that would have been excluded from treatment with chemotherapy. For instance, this risk has been recently showed, for ICIs, in the setting of advanced urothelial cancer: after approval of ICIs in clinical practice, initiation of ICIs near the end of life significantly increased among patients with poor performance status, while did not significantly change among individuals with good performance status [43]. Again, in our study the multivariate analysis confirmed ECOG PS as prognostic factors, suggesting that this important factor drives OS.

Sex is one of the factors included in the DiM prognostic score. Traditionally, in the chemotherapy era, several analyses showed that males performed slightly worse compared to females [44]. Nevertheless, a recently published meta-analysis of trials testing ICIs reported a significant interaction between ICIs efficacy and gender, with worse outcome in females, probably due to a high occurrence of driver mutations [45]. However, another recent meta-analysis in 23 randomized trials in ICIs-treated patients did not reported differences among sex [46].

Usually, patients harbouring squamous-NSCLC and especially those with rare histotypes (large cell neuroendocrine carcinoma, mixed and undifferentiated carcinoma) had a poor prognosis compare to adenocarcinoma [47]. Despite its negative prognostic role, the squamous histology seems to highly benefit from ICIs [4,5] as well as adenocarcinoma patients, while in rare histologies the ICIs role remain unclear [48].
In the study by Di Maio et al., based on patients treated several years ago, patients who received first-line platinum-based therapy had a worse outcome with second-line therapy. However, this is difficult to be applied in patients treated with ICIs, considering that nowadays the majority of patients receive first-line platinum-based chemotherapy [38]. However, the response to previous therapies seems to correlate with response to ICIs in a retrospective analysis [26].
Ideally, the identification of predictive factors could improve decision making in clinical practice. With all the limitations of this indirect comparison, the role of ICIs in improving OS in aNSCLC could be observed when we compare mOS of B and I categories in patients treated with ICIs in our present series with patients treated with CHT in the original development of the DiM score: 18.0 vs 12.9 mo and 8.5 vs 6.9 mo for B and I category, respectively. Hence, when we compare W category results (4.0 vs 2.6 mo) ICIs perform worse in terms of OS compare to CHT, possibly due to a detrimental effect of ICIs in this group of patients. However, patients included in the present analysis were treated in clinical practice, and probably this allowed the inclusion of some patients that, due to poor performance status, were excluded from the clinical trials with docetaxel, used for the development of DiM score. This could partially explain the worse outcome of the W category in the present series. Consequently, this indirect comparison does not allow a robust definition of the absolute benefit associated with ICIs in the different prognostic groups. Furthermore, it is important to emphasize that the score remains prognostic rather than predictive. Formally, despite the poor outcome, we cannot exclude that ICIs is associated with activity and efficacy also in the group of patients with worse prognosis. However, the absolute outcomes in that group are undoubtedly poor, and an honest and serious reflection should be made on the cost-effectiveness of treatment with ICIs in these patients.
In addition to clinical factors [50], multiple inflammatory markers have been recently investigated as possible

prognostic and predictive biomarkers, due to their easy accessibility and limited costs. The role of peripheral immune cells, through routine blood parameters, was recently studied in patients treated with ICIs [51,52]. Neutrophil-to-lymphocyte ratio (NLR) is the most studied, because it better reflects the balance between pro-tumour and anti-tumour activity of the host immune system [53,54]. Both, Jiang et al and Cao et al. reported that higher baseline and post-treatment NLR was associated with poor PFS and OS [55,56].

The assessment of different biomarkers in a single predictive/prognostic score can allow to identify patients who mostly have a survival benefit from ICIs. Many immune-based scores were studied using clinical characteristics and blood biomarkers such as "immunotherapy Sex - ECOG - NLR - Delta NLR" iSEND [11], "Advanced Lung cancer inflammation Index" (ALI) [18], "Lung Immune Prognostic Index" LIPI [24], "Systemic Inflammation Index" SII [27] and "Aggregate Index of Systemic Inflammation" AISI [32]. All these scores included NLR and most of them LDH and ECOG-PS [57-60]. Similarly, to our study they identified different predictive/prognostic groups which are statistically significantly associated with a progressive worse PFS or OS. As confirmed in our multivariate analysis also baseline liver metastases and heavy smokers (≥40 pack/years) seems to play a relevant role in survival. This was recently demonstrated also in recent a study from our group: a score called EPSILoN was created and then validated including 5 different prognostic parameters such as NLR, LDH, smoke, ECOG PS and liver metastases [59].

The major limitation of our study, as other recent papers which tried to propose prognostic/predictive scores in patients treated with ICIs, is its retrospective nature, without a control group of patients not receiving ICIs. The control arm is necessary to assess the real predictive role of a marker/score. Moreover, the study lacks the PD-L1 status of patients included in the analysis because for some patients receiving ICIs within Expanded Access Program, PD-L1 test ere not required, especially in the beginning, and so its correlation with clinical factors is impossible. Moreover, when we compare the population between the two centers some statistically significant differences exist, e.g.: in C1 more female were included compare to C2, this probably because smoking habits it's more diffuse in more emancipated countries like North Italy (C1) compare to South (C2). This could be also the reason for treating more stage III patients in C2 compare to C1. Another difference was seen among

patients with ECOG-PS 2 which is more represented; this probably due to the more accurate selection of patients within clinical trial (more frequent in C1) compare to those included in the Expanded Access Program and less clinical trial (C2). Adenocarcinoma was most frequent in C1 compare to C2 this probably because in C1 were included more female patients with a younger median age (65 vs 67 years), the latter can also be the reason of why patients presents with more CNS, liver and bone metastases at baseline ICIs.

Finally, to our knowledge, this is the first study which, applying a score originally developed in patients receiving chemotherapy in a series of patients receiving immunotherapy, allows an indirect comparison between these 2 treatments in different prognostic groups.

Finally, given their easy use, this score could be readily integrated into routine clinical practice helping clinicians in decision-making.

5. Conclusions

DiM score, generated in patients treated with 2L CHT, is able to predict prognosis also in patients treated with ICIs. However, its value remain prognostic and not predictive to immunotherapy. These results showed that patients within the worst category (bad clinical factors) has a short absolute life expectancy, and probably would not benefit from any active systemic therapy, regardless of treatment type. Reasonably, for this subset of patients, best supportive care could be the best choice. In any case, integrating a composite biomarker (clinical and laboratoristic factors with molecular and PD-L1 status) is necessary for these patients, since ICIs could even have a detrimental effect.

Abbreviations

Immune checkpoint inhibitors, ICIs; advanced non-small cell lung cancer, aNSCLC; programmed-death ligand-one, PD-L1; overall survival, OS; patients, pts; immunotherapy, IO; progression-free survival, PFS; second-line,

2L; months, mo; Eastern Cooperative Oncology Group Performance Status, ECOG-PS; centre 1, C1; centre 2 C2; chemotherapy, CHT; intravenously, IV; progressive disease, PD; median PFS, mPFS; median OS, mOS; total body computed tomography, TB CT; Response Evaluation Criteria in Solid Tumours, RECIST; scan Overall response rate, ORR; complete response, CR; partial response, PR; disease control rate, DCR; stable disease , SD; confidence interval, CI; best category, B; intermediate category, I; worst category, W; hazard ratio, HR; concordance statistic, C-index; Neutrophil-to-lymphocyte ratio, NLR;

Acknowledgements

We would like to thank our nurse assistant: Anna Maria Leone for her support.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

FdB: provided consultation, attended advisory boards and/or provided lectures for the following organizations, from whom received honoraria or education grants: Amgen, AstraZeneca, Boehringer-Ingelheim, BMS, Eli Lilly, F. Hoffmann-La Roche, Ignyta, Merck Sharp and Dohme, Merck Serono, Novartis, Pfizer.

MG: declares personal financial interests with the following organizations: AstraZeneca, MSD International GmbH, BMS, Boehringer Ingelheim Italia S.p.A, Celgene, Eli Lilly, Ignyta, Incyte, Inivata, MedImmune, Novartis, Pfizer, Roche, Takeda; she also declares Institutional financial interests with the following organizations: Eli Lilly, MSD, Pfizer (MISP), AstraZeneca, MSD International GmbH, BMS, Boehringer Ingelheim Italia S.p.A, Celgene, Ignyta, Incyte, Inivata, MedImmune, Novartis, Pfizer, Roche, Takeda, Tiziana, Foundation Medicine; at

the end, she has received research funding from the following organizations: AIRC, AIFA, Italian Moh, TRANSCAN.GLR declares personal fees from Eli Lilly, BMS and AstraZeneca, outside the submitted work. CP declares personal fees from BMS and MSD, outside the submitted work. MCG declares personal fees from MSD, AstraZeneca, Eli Lilly and BMS, outside the submitted work. DS declares personal fees from AstraZeneca, Boehringer Ingelheim and BMS, outside the submitted work.

The other authors report no conflict of interest.

References

1. Califano R, Kerr K, Morgan RD et al. Immune Checkpoint Blockade: A New Era for Non-Small Cell Lung Cancer. Curr Oncol Rep. 2016 Sep;18(9):59.

2. Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. N Engl J Med. 2016;375(19):1823-1833.

3. Proto C, Ferrara R, Signorelli D, et al. Choosing wisely first line immunotherapy in non-small cell lung cancer (NSCLC): what to add and what to leave out. Cancer Treat Rev. 2019 May;75:39-51

4. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med 2015;373:123–135.

5. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-

Small-Cell Lung Cancer. N Engl J Med 2015;373:1627–139.

6. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): A randomised controlled trial. Lancet 2016;387:1540–1550.

7. Rittmeyer A, Barlesi F, Waterkamp D, et al. Atezolizumab versus docetaxel in patients with previously treated non-small cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. Lancet 2017;389:255-265.

8. Garassino MC, Cho BC, Kim JH, et al. ATLANTIC Investigators. Durvalumab as third-line or later treatment for advanced non-small-cell lung cancer (ATLANTIC): an open-label, single-arm, phase 2 study. Lancet Oncol. 2018 Apr;19(4):521-536.

9. Mukherji D, Jabbour MN, Saroufim M, et al. Programmed death-Ligand 1 expression in muscle-Invasive bladder cancer cystectomy specimens and lymph node metastasis: a reliable treatment selection biomarker? Clin Genitourin Canc 2016;14:183e7.

10. Chae YK, Pan A, Davis AA, et al. Biomarkers for PD-1/PD-L1 blockade therapy in non-small-cell lung cancer: is PD-L1 expression a good marker for patient selection? Clin Lung Canc 2016;17: 350e61.

11. Park W, Kwon D, Saravia D, et al. Developing a Predictive Model for Clinical Outcomes of Advanced Non-Small Cell Lung Cancer Patients Treated With Nivolumab. Clin Lung Cancer. 2018;19(3):280-288.

12. Tay R, Prelaj A, Califano R. Immune checkpoint blockade for advanced non-small cell lung cancer: challenging clinical scenarios. J Thorac Dis. 2018 May;10(Suppl 13):S1494-S1502.

13. Fucà G, Galli G, Poggi M, et al. Modulation of peripheral blood immune cells by early use of steroids and its association with clinical outcomes in patients with metastatic non-small cell lung cancer treated with immune checkpoint inhibitors. ESMO Open. 2019 Feb 27;4(1):e000457

14. Fucà G, Galli G, Poggi M, et al. Low Baseline Serum Sodium Concentration Is Associated with Poor Clinical Outcomes in Metastatic Non-Small Cell Lung Cancer Patients Treated with Immunotherapy. Target Oncol. 2018 Dec;13(6):795-800.

15. Giulia G, Tiziana T, Claudia P, et al. Association between antibiotic-immunotherapy exposure ratio and outcome in metastatic non-small cell lung cancer. Lung Cancer, Volume 132, 72 – 78

16. Prelaj A, Tay R, Ferrara R, et al. Predictive biomarkers of response for immune checkpoint inhibitors in non-small-cell lung cancer. Eur J Cancer. 2019 Jan;106:144-159. doi: 10.1016/j.ejca.2018.11.002. Epub 2018 Dec 5. Review

17. Templeton AJ, Ace O, McNamara MG, et al. Prognostic role of platelet to lymphocyte ratio in solid tumors: a systematic review and meta-analysis. Cancer Epidemiol Biomarkers Prev 2014;23(7):1204-1212.

18. Shiroyama T, Suzuki H, Tamiya M, et al. Pretreatment advanced lung cancer inflammation index (ALI) for predicting early progression in nivolumab- treated patients with advanced non–small cell lung cancer. Cancer Med 2018;7(1):13-20.

19. Russo A, Franchina T, Ricciardi GRR, et al. Baseline neutrophilia, derived neutrophil-to-lymphocyte ratio (dNLR), platelet-to-lymphocyte ratio (PLR), and outcome in non-small cell lung cancer (NSCLC) treated with Nivolumab or Docetaxel. J Cell Physiol. 2018;233(10):6337-6343.

20. Ferrucci PF, Ascierto PA, Pigozzo J, et al. Baseline neutrophils and derived neutrophil-to-lymphocyte ratio: prognostic relevance in metastatic melanoma patients receiving ipilimumab. Ann Oncol. 2016;27(4):732-738.

21. Weide B, Martens A, Hassel JC, et al. Baseline biomarkers for outcome of melanoma patients treated with pembrolizumab. Clin Cancer Res. 2016;22(22):5487-5496.

22. Bagley SJ, Kothari S, Aggarwal C, et al. Pretreatment neutrophil-to-lymphocyte ratio as a marker of outcomes in nivolumab-treated patients with advanced non-small-cell lung cancer. Lung Cancer. 2017;106:1-7.

23. Diem S, Schmid S, Krapf M, et al. Neutrophil-to-Lymphocyte ratio (NLR) and Platelet-to-Lymphocyte ratio (PLR) as prognostic markers in patients with non-small cell lung cancer (NSCLC) treated with nivolumab. Lung Cancer. 2017;111:176-181.

24. Mezquita L, Auclin E, Ferrara R, et al. Association of the Lung Immune Prognostic Index With Immune Checkpoint Inhibitor Outcomes in Patients With Advanced Non-Small Cell Lung Cancer. JAMA Oncol 2018;4(3):351-357.

25. Nakaya A, Kurata T, Yoshioka H, et al. Neutrophil-to-lymphocyte ratio as an early marker of

outcomes in patients with advanced non-small-cell lung cancer treated with nivolumab. Int J Clin Oncol. 2018;23(4):634-640.

26. Garde-Noguera J, Martin-Martorell P, De Julián M et al. Predictive and prognostic clinical and pathological factors of nivolumab efficacy in non-small-cell lung cancer patients. Clin Transl Oncol. 2018;20(8):1072-1079.

27. Suh KJ, Kim SH, Kim YJ, et al. Post-treatment neutrophil-to-lymphocyte ratio at week 6 is prognostic in patients with advanced non-small cell lung cancers treated with anti-PD-1 antibody. Cancer Immunol Immunother. 2018;67(3):459-470.

28. Tanizaki J, Haratani K, Hayashi H, et al. Peripheral Blood Biomarkers Associated with Clinical Outcome in Non – Small Cell Lung Cancer Patients Treated with Nivolumab. J Thorac Oncol. 2018;13(1):97-105.

29. Di Maio M, Lama N, Morabito A, et al. Clinical assessment of patients with advanced non-small-cell lung cancer eligible for second-line chemotherapy: a prognostic score from individual data of nine randomised trials. Eur J Cancer. 2010 Mar;46(4):735-4

30. Di Maio M, Krzakowski M, Fougeray R, et al. Prognostic score for second-line chemotherapy of advanced non-small-cell lung cancer: external validation in a phase III trial comparing vinflunine with docetaxel. Lung Cancer. 2012 Jul;77(1):116-20

31. Schemper M1, Smith TL. A note on quantifying follow-up in studies of failure time. Control Clin Trials. 1996 Aug;17(4):343-6.

32. Royston P, Parmar MK, Sylvester R. Construction and validation of a prognostic model across several studies, with an application in superficial bladder cancer. Stat Med 2004;23:907–26

33. Pencina MJ, D'Agostino RB. Overall C as a measure of discrimination in survival analysis: model specific population value and confidence interval estimation. Stat Med 2004;23:2109–23.R

34. Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.2018. URL https://www.R-project.org/.

35. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45(2), 228-247.

36. Ferrara R, Mezquita L, Texier M, et al. Hyperprogressive Disease in Patients With Advanced Non-Small Cell Lung Cancer Treated With PD-1/PD-L1 Inhibitors or With Single-Agent Chemotherapy. JAMA Oncol. 2018 Nov 1;4(11):1543-1552

37. Champiat S, Ferrara R, Massard C, et al. Hyperprogressive disease: recognizing a novel pattern to improve patient management.Nat Rev Clin Oncol. 2018 Dec;15(12):748-762.

38. Lo Russo G, Moro M, Sommariva M, et al. Antibody-Fc/FcR Interaction on Macrophages as a Mechanism for Hyperprogressive Disease in Non-small Cell Lung Cancer Subsequent to PD-1/PD-L1 Blockade. Clin Cancer Res. 2019 Feb 1;25(3):989-999

39. Putzu C, Cortinovis DL, Colonese F, et al. Blood cell count indexes as predictors of outcomes in advanced non-small-cell lung cancer patients treated with Nivolumab. Cancer Immunol Immunother. 2018;67(9):1349-1353.

40. Tamiya M, Tamiya A, Inoue T, et al. Metastatic site as a predictor of nivolumab efficacy in patients with advanced non-small cell lung cancer: A retrospective multicenter trial PLoS One. 2018;13(2):e0192227.

41. Middleton G, Brock K, Summers Y, et al. Pembrolizumab in performance status 2 patients with non-

small cell lung cancer (NSCLC): Results of the PePS2 trial. Annals of Oncology 2018;29 (suppl_8):viii493-viii547.

42. Passaro A, Spitaleri G, Gyawali B, et al. Immunotherapy in Non-Small-Cell Lung Cancer Patients With Performance Status 2: Clinical Decision Making With Scant Evidence. J Clin Oncol. 2019 Apr 17:JCO1802118

43. Parikh RB, Galsky MD, Gyawali B, et al. Trends in Checkpoint Inhibitor Therapy for Advanced Urothelial Cell Carcinoma at the End of Life: Insights from Real-World Practice. Oncologist. 2019 Apr 3. pii: theoncologist.2019-0039.

44. Radzikowska E, Głaz P, Roszkowski K. Lung cancer in women: age, smoking, histology, performance status, stage, initial treatment and survival. Population-based study of 20 561 cases. Ann Oncol 2002;13:1087–93.

45. Conforti F, Pala L, Bagnardi V, et al. Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis. Lancet Oncol. 2018 Jun;19(6):737-746. doi: 10.1016/S1470-2045(18)30261-4. Epub 2018 May 16.

46. Christopher J. D.Wallis, Mohit Butaney, Raj Satkunasivam, et al. Association of Patient Sex With Efficacy of Immune Checkpoint Inhibitors and Overall Survival in Advanced Cancers. A Systematic Review and Meta-analysis. JAMA Oncol. 2019;5(4):529-536.

47. Pirker R, Pereira JR, Szczesna A, et al. Prognostic factors in patients with advanced non-small cell lung cancer: data from the phase III FLEX study. Lung Cancer. 2012 Aug;77(2):376-82.

48. Signorelli D, Ferrara R, Proto C, et al. Immune-checkpoints inhibitors in metastatic non-small cell lung cancer with rare histology. Abstract#9106 (270539) (Poster at ASCO 2019)

49. Weiss GJ, Rosell R, Fossella F, et al. The impact of induction chemotherapy on the outcome of second-line therapy with pemetrexe or docetaxel in patients with advanced non-small-cell lung cancer. Ann Oncol 2007;18:453–60

50. Schmid S, Diem S, Li Q, et al. Organ-specific response to nivolumab in patients with non-small cell lung cancer (NSCLC). Cancer Immunol Immunother. 2018;67(12):1825-1832.

51. Prelaj A, P. Pizzutilo, M. Montrone, et al. Prognostic and Predictive Role of Peripheral Blood Biomarkers in NSCLC Patients Treated with Checkpoint, a Single-Center Experience. JTO Vol 13, Issue 10, Suppl, Page S926.

52. Boeri M, Milione M, Proto C, et al. Circulating miRNAs and PD-L1 Tumor Expression Are Associated with Survival in Advanced NSCLC Patients Treated with Immunotherapy: a Prospective Study. Clin Cancer Res. 2019 Apr 1;25(7):2166-2173

53. Zer A, Sung MR, Walia P, et al. Correlation of Neutrophil to Lymphocyte Ratio and Absolute Neutrophil Count With Outcomes With PD-1 Axis Inhibitors in Patients With Advanced Non-Small-Cell Lung Cancer. Clin Lung Cancer. 2018;19(5):42

54. Fukui T, Okuma Y, Nakahara Y, et al. Activity of Nivolumab and Utility of Neutrophil-to-Lymphocyte Ratio as a Predictive Biomarker for Advanced Non-Small-Cell Lung Cancer: A Prospective Observational Study. Clin Lung Cancer. 2018;pii: S1525-7304(18)30103-7.

55. Jiang T, Qiao M, Zhao C, et al. Pretreatment neutrophil-to-lymphocyte ratio is associated with outcome of advanced-stage cancer patients treated with immunotherapy: a meta-analysis. Cancer Immunol Immunother. 2018;67(5):713-727.

56. Cao D, Xu H, Xu X, et al. A reliable and feasible way to predict the benefits of Nivolumab in patients with non-small cell lung cancer: a pooled analysis of 14 retrospective studies. Oncoimmunology. 2018;7(11):e150726

57. Cona MS, Lecchi M, Cresta S, et al. Combination of Baseline LDH, Performance Status and Age as Integrated Algorithm to Identify Solid Tumor Patients with Higher Probability of Response to Anti PD-1 and PD-L1 Monoclonal Antibodies. Cancers (Basel). 2019 Feb 14;11(2).

58. Prelaj A, Sara SE, Pizzutilo P, et al. Predictive score using clinical and blood biomarkers in advanced non small cell lung cancer (aNSCLC) patients treated with immunotherapy. Ann Oncology, Vol 29, suppl_10, Dec 2018.

59. Prelaj A, Ferrara R, Rebuzzi SE, Proto C, Signorelli D, Galli G, De Toma A, Randon G, Pagani F, Viscardi G, et al. EPSILoN: A Prognostic Score for Immunotherapy in Advanced Non-Small-Cell Lung Cancer: A Validation Cohort. Cancers (Basel). 2019 Dec 5;11(12). pii: E1954. doi: 10.3390/cancers11121954.

60. Signorelli D, Giannatempo P, Grazia G, et al., “Patients Selection for Immunotherapy in Solid Tumors: Overcome the Naïve Vision of a Single Biomarker,” BioMed Research International, vol. 2019, Article ID 9056417, 15 pages, 2019.

Table legends

Table 1. Patients characteristics in the two participating Institutions and in the whole series.

Table 2. Description of the scoring system [29-30].

Table 3. Multivariate analysis according to OS for overall population and baseline patient's characteristics.

Figure legends

Figure 1. Kaplan-Meier curve of overall survival for three different prognostic groups.

Figure 2. Kaplan-Meier curves of progression-free survival in the three different prognostic groups.

Appendix

Figure 1 Appendix. Kaplan-Meier curve of progression-free survival for Center 1 (A) and Center 2 (B)

Figure 2 Apendix. Kaplan-Meier curves of overall survival for Center 1 (A) and Center 2 (B)